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Research article

Qualitative and quantitative analysis of the saponins in *Panax notoginseng* leaves using ultra-performance liquid chromatography coupled with time-of-flight tandem mass spectrometry and high performance liquid chromatography coupled with UV detector

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ABSTRACT

Background: *Panax notoginseng* leaves (PNL) exhibit extensive activities, but few analytical methods have been established to exclusively determine the dammarane triterpene saponins in PNL.
Methods: Ultra-performance liquid chromatography coupled with time-of-flight mass spectrometry (UPLC/Q-TOF MS) and HPLC-UV methods were developed for the qualitative and quantitative analysis of ginsenosides in PNL, respectively.
Results: Extraction conditions, including solvents and extraction methods, were optimized, which showed that ginsenosides Rc and Rb3, the main components of PNL, are transformed to notoginsenosides Fe and Fd, respectively, in the presence of water, by removing a glucose residue from position C-3 via possible enzymatic hydrolysis. A total of 57 saponins were identified in the methanolic extract of PNL by UPLC/Q-TOF MS. Among them, 19 components were unambiguously characterized by their reference substances. Additionally, seven saponins of PNL—ginsenosides Rb1, Rc, Rb2, and Rb3, and notoginsenosides Fc, Fe, and Fd—were quantified using the HPLC-UV method after extraction with methanol. The separation of analytes, particularly the separation of notoginsenoside Fc and ginsenoside Rc, was achieved on a Zorbax ODS C8 column at a temperature of 35°C. This developed HPLC-UV method provides an adequate linearity ($r^2 > 0.999$), repeatability (relative standard deviation, RSD < 2.98%), and inter- and intraday variations (RSD < 4.40%) with recovery (98.7–106.1%) of seven saponins concerned. This validated method was also conducted to determine seven components in 10 batches of PNL.
Conclusion: These findings are beneficial to the quality control of PNL and its relevant products.

1. Introduction

Panax notoginseng (Burk) F. H. Chen, radix has been traditionally applied to treat hematological diseases and cardiovascular diseases in China since ancient times [1,2]. The main bioactive components of *P. notoginseng* are identified as drammarane triterpenoid saponins, including 20(*S*)-protopanaxatriol-type (PTS) and 20(*S*)-protopanaxadiol-type saponins (PDS), which are present in different parts of the plant, including its roots, rhizomes, leaves, and flower buds [3,4]. *P. notoginseng* roots are commonly used in remedies for various ailments. Because of the strict environmental conditions required for its growth and increased usage, the supply of roots of *P. notoginseng* could hardly meet the rapidly increasing market demands in recent years. Annual recovery of the leaves might be a feasible alternative source of ginsenosides, compared to the 3-yr growth cycle of the roots [1]. Our previous study has indicated that the chemical characteristics of the leaves and roots of *P. notoginseng* are significantly different [4]. *P. notoginseng* leaves (PNL) contain an abundance of PDS, such as notoginsenosides Fc and ginsenosides Rc, Rb2, and Rb3, which are rarely found in the roots [3,4].

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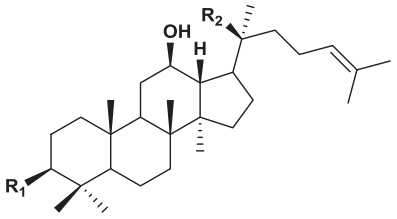

20(S)-Protopanaxadiol type

No.	Compounds	R_1	R_2
P1	Ginsenoside Rb_1	-Oglc(2-1)glc	-Oglc(6-1)glc
P2	Notoginsenoside Fc	-Oglc(2-1)glc(2-1)xyl	-Oglc(6-1)xyl
P3	Ginsenoside Rc	-Oglc(2-1)glc	-Oglc(6-1)araf
P4	Ginsenoside Rb_2	-Oglc(2-1)glc	-Oglc(6-1)arap
P5	Ginsenoside Rb_3	-Oglc(2-1)glc	-Oglc(6-1)xyl
P6	Notoginsenoside Fe	-Oglc	-Oglc(6-1)araf
P7	Notoginsenoside Fd	-Oglc	-Oglc(6-1)xyl

Fig. 1. Chemical structures of seven investigated saponins from *Panax notoginseng* leaves. Araf, α-L-arabinose (furanose); Arap, α-L-arabinose (pyranose); Glc, β-D-glucose; Xyl, β-D-xylose.

PNL, traditionally used in the treatment of insomnia, have a strong neuroprotective activity [5], antidepressant effects [6], and protective effects against alcoholic liver injury [7]. In recent years, most studies focused on the roots of *P. notoginseng*, and little attention was paid to its leaves. Few analytical methods, including high performance liquid chromatography (HPLC)-UV [8,9] and HPLC-mass spectrometry (MS) [3,10], have been developed to exclusively determine ginsenosides in PNL. However, in most of these studies, notoginsenoside Fc and ginsenoside Rc, the two major components of PNL, were not well separated, leading to the inaccurate quantitation. Furthermore, water was used as the solution in sample preparation [9]. Our preliminary data have demonstrated that the severe transformation of ginsenosides Rc, Rb2, and Rb3 occurred when PNL was extracted with water, which might not reflect the real chemical profile of PNL. The aim of present study, therefore, is to develop ultra-performance liquid chromatography coupled with time-of-flight MS (UPLC/Q-TOF MS) and HPLC-UV methods designed specifically for the qualitative and quantitative determination of saponins in PNL, respectively.

2. Materials and methods

2.1. Chemicals and materials

Ten batches of PNL were obtained from Yunnan province, China. Their botanical origin of materials was identified by Professor Ni Ma of Wenshan Sanqi Institute of Science and Technology, Wenshan University, China. Authentic standards, including ginsenosides Rb1, F1, F2, and notoginsenoside Fe, were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); ginsenosides Rg1, Rb2, Rb3, Rc, Re, and notoginsenoside R1 were supplied by MUST Bio-technology Co., Ltd. (Chengdu, China); ginsenosides Rg3 and Rd were purchased from Zelang Medical Technology Co., Ltd. (Beijing, China); ginsenosides Mc and Rk1, compound K, notoginsenosides Fc and Fd, gypenoside XIII, and vinaginsenoside R7 were isolated previously from the roots and leaves of *P. notoginseng*. The purity of all standards used in the study was more than 95%, detected by HPLC-UV analysis. All voucher specimens are stocked at the Institute of Chinese Medical Sciences, University of Macau, Macao, China. HPLC-grade acetonitrile, methanol, and formic acid were supplied by Merck (Darmstadt, Germany); the deionized water was prepared using a Milli-Q Integral Water Purification System (Millipore, Bedford, MA, USA).

2.2. Preparation of standard solutions

For qualitative and quantitative analysis, two sets of mixed standards containing all 19 saponins and seven saponins (i.e., ginsenosides Rb1, Rc, Rb2, and Rb3, and notoginsenosides Fc, Fe, and Fd; Fig. 1) were dissolved in methanol, respectively.

2.3. Pressurized liquid extraction

Pressurized liquid extraction (PLE) was conducted on a Dionex ASE 300 system (Dionex, Sunnyvale, CA, USA). To reduce the solvent volume, diatomaceous earth is recommended to use in PLE as a dispersion agent. First, 500 mg of PNL fine powder (0.074–0.10 mm) was mixed with the equal weight of diatomaceous earth, and extracted using the following conditions: methanol as solvent; 140°C of extraction temperature; 6.89×10^3 kPa of pressure; 15 min of static extraction time; one of number of extraction; 60% of the flush volume. The PLE extract (~22 mL) was transferred into a 25-mL volumetric flask, which was brought up to its volume with the same solvent and then filtered through a MILLEX-GV (0.22 μm, PVDF membrane; Millipore, Carrigtwohill, Cork, Ireland) prior to HPLC-UV and UPLC/Q-TOF MS analysis.

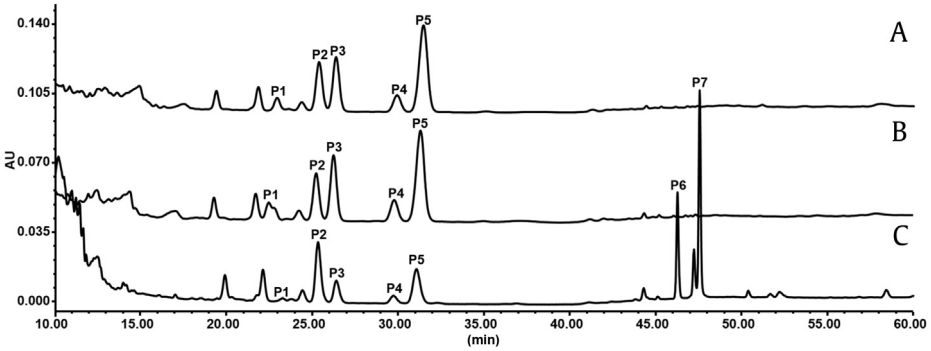

Fig. 2. HPLC-UV chromatograms of the extracts of *Panax notoginseng* leaves by pressurized liquid extraction. (A) Methanolic extract. (B) Ethanolic extract. (C) Aqueous extract. HPLC, high performance liquid chromatography; P1, ginsenoside Rb1; P2, notoginsenoside Fc; P3–P5, ginsenosides Rc, Rb2, and Rb3; P6–P7, notoginsenosides Fe and Fd.

2.4. HPLC-UV analysis

HPLC analysis was conducted on an Alliance e2695 HPLC system (Waters, Milford, MA, USA), equipped with a quaternary solvent delivery system, a vacuum degasser, an autosampler, and a Waters 2996 photodiode array detector. The separation was conducted on a Zorbax ODS C8 column (250 mm × 4.6 mm i.d., 5 μm, Agilent, Santa Clara, CA, USA) at a flow rate of 0.8 mL/min. A gradient elution system consisted of water (A) and acetonitrile (B) using the following gradient program: 0–5 min, 15–30% B; 5–15 min, 30–32% B; 15–35 min, 32–32% B; 35–45 min, 32–45% B; 45–60 min, 45–50% B. Column temperature and the detection wavelength were set 35°C and 203 nm, respectively.

2.5. UPLC/Q-TOF MS analysis

Qualitative analysis of saponins in PNL was performed using a Waters ACQUITY UPLC H-Class system coupled with a Waters SYNAPT G2-Si Q-TOF mass spectrometer (Waters, Manchester, UK). An ACQUITY HSS T3 column (100 mm × 2.1 mm, 1.8 μm, Waters, Milford, MA, USA) at 45°C was used. The mobile phase contained 0.1% aqueous formic acid (A) and acetonitrile (B) at 0.4 mL/min flow rate under the following gradient program: 0–5 min, 20–33% B; 5–12 min, isocratic 33% B; 12–20 min, 33–100% B. The eluent was subjected directly to a Q-TOF MS by an electrospray ionization interface (Waters, Manchester, UK), which was operated in negative ion mode. The capillary voltage was 2.5 kV, and the source temperature and desolvation temperature were set at 120°C and 450°C, respectively. The cone gas flow and nebulization gas flow were set at 10 L/h and 600 L/h, respectively. The scan time for the Quan-qual MS^E method was set to 0.2 s, and the trap collision energy was ramped from 20 eV to 80 eV. MS data were acquired in centroid mode from m/z 50 to m/z 1,500 in full scan using the MassLynx software (Waters, Manchester, UK). Accurate mass was maintained by continuously infusing leucine-enkephalin calibrant (200 pg/mL) at 10 μL/min by LockSpray interface, producing the reference ion ($[M\text{-}H]^- = 554.2615$).

2.6. Linearity, limit of detection, and limit of quantification

A series of appropriate concentrations of working solutions containing ginsenosides Rb1, Rc, Rb2, and Rb3, and notoginsenosides Fc, Fe, and Fd, were prepared by the dilution of methanol stock solution for HPLC-UV analysis. Six concentrations of the seven saponins were determined under the same chromatographic conditions, and their calibration curves were obtained by plotting the peak area versus the corresponding concentration of saponin concerned. The limit of quantification (LOQ) and limit of detection (LOD) of each saponin were measured at a signal/noise ratio of about 10 and 3, respectively, under same chromatographic conditions.

2.7. Precision, repeatability, stability, and recovery

Inter- and intraday precisions were determined in sample PNL10 six times within a single day and 3 consecutive days for

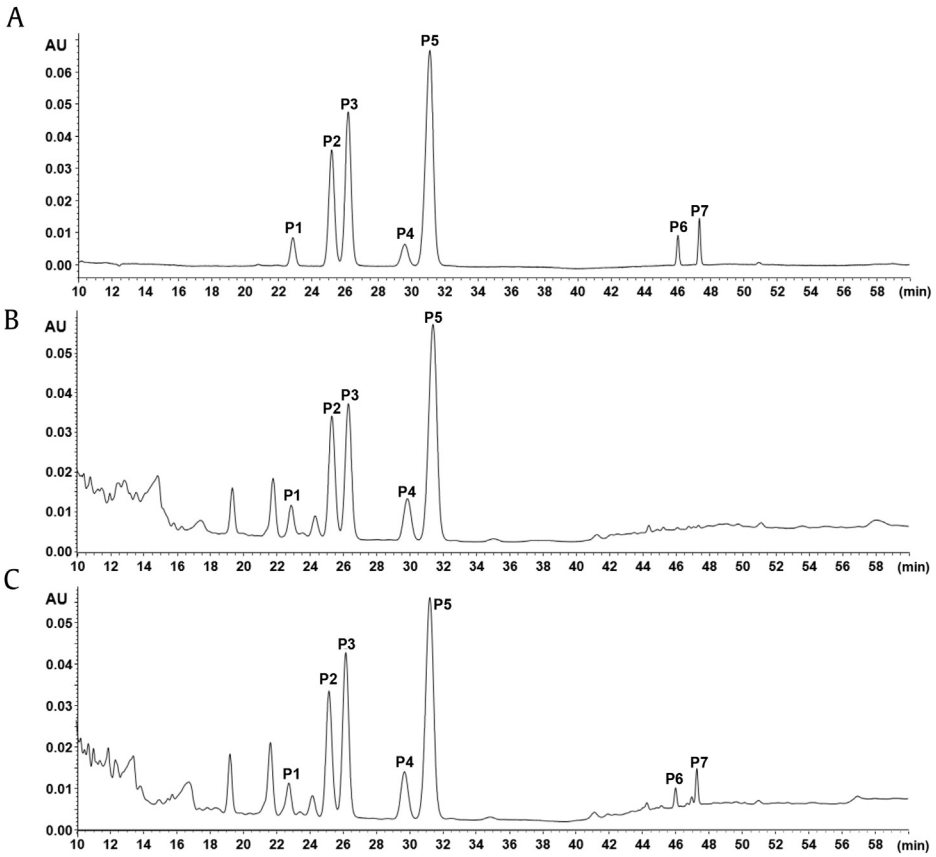

Fig. 3. HPLC-UV chromatograms. (A) Mixed standards. (B) PLE extract of *Panax notoginseng* leaves (sample PNL01). (C) PLE extract of *P. notoginseng* leaves (sample PNL07). HPLC, high performance liquid chromatography; P1, ginsenoside Rb1; P2, notoginsenoside Fc; P3–P5, ginsenosides Rc, Rb2, and Rb3; P6–P7, notoginsenosides Fe and Fd; PLE, pressurized liquid extraction.

every analyte, respectively. To test its repeatability, sample PNL10 was parallelly divided into six parts and extracted by PLE, and analyzed by HPLC-UV as mentioned above. To examine the inherent stability characteristics of the tested saponins, the freshly prepared sample PNL10 was stocked at room temperature, and was analyzed at different time intervals of 0 h, 6 h, 12 h, and 24 h. Variation was expressed as relative standard deviation.

A recovery test for the assessment of accuracy was performed using the standards addition method. Three concentration levels of seven authentic standards (approximately equivalent to 80%, 100%, and 120% levels of each compound) were added into the sample of PNL10. The spiked samples were extracted and analyzed by HPLC-UV. Each set of addition was repeated three times. The extraction recovery was calculated as follows:

$$\text{Recovery (\%)} = (\text{Amount determined} - \text{Amount original})/\text{Amount spiked} \times 100.$$

3. Results and discussion

3.1. Optimization of extraction solvents

The choice of extraction solvents is important to extract the target chemicals from the sample matrix, and will significantly influence the yields and variety of constituents present in herbal medicines. Three common solvents—methanol, ethanol, and distilled water—were used to extract the saponins from PNL by PLE. Interestingly, the HPLC profiles of aqueous and alcohol extracts are remarkably different (Fig. 2). Compared to either methanolic or ethanolic extract, the contents of ginsenosides Rc (P3) and Rb3 (P5) were markedly decreased in the aqueous extract. More importantly, four peaks that did not present in alcohol extracts were observed at 40–50 min in aqueous extract (Fig. 2), suggesting that new chemical components are produced using water as the extraction solvent. Among these new components, two major peaks (P6 and P7) were identified as notoginsenosides Fe and Fd by comparing their retention behavior with those of the authentic standards, which was also confirmed by LC-MS analysis. In order to investigate whether this change was caused by PLE, the other extraction methods, such as ultrasonic and reflux extractions, were also conducted. As shown in Fig. S1, similar changes were observed in aqueous extracts regardless of the extraction method used.

A most plausible explanation is that ginsenoside transformation occurs during extraction with water as solvent. Ginsenosides Rc and Rb_3, the major components of PNL, are structurally similar to notoginsenosides Fe and Fd, respectively, but have two glucose residues [β-D-glucopyranosyl-(1→2)-β-D-glucopyranose] at position C-3, rather than one (Fig. 1). Therefore, ginsenosides Rc and Rb_3 can easily be transformed to notoginsenosides Fe and Fd, respectively, by removing a glucose residue from position C-3 via mild acid hydrolysis or enzymatic hydrolysis [11,12]. Neither acid nor base was added in the extraction solvent; as such, it was reasonable to presume that these transformations were driven by the enzyme present in PNL. Thus, water is not suitable to extract the saponins from PNL, although it can avoid the influence of pigments that are abundant in plant leaves. Because of the high extraction efficiency and repeatability [4,13], PLE with methanol solvent was selected for sample preparation.

3.2. Optimization of HPLC-UV conditions

To achieve the high-resolution separation of saponins from PNL, the HPLC chromatographic conditions, such as mobile phase, column, and column temperature, were systematically optimized in the pilot study. Several previous studies showed that notoginsenoside Fc and ginsenoside Rc, two main components of PNL, could not be well separated [8,9]. Therefore, three chromatographic columns, including Agilent Zorbax ODS C18 (250 mm × 4.6 mm i.d., 5 μm, Santa Clara, CA, USA) column, Zorbax ODS C8 (250 mm × 4.6 mm i.d., 5 μm, Santa Clara, CA, USA) column, and Waters Atlantis T3 (250 mm × 4.6 mm i.d., 5 μm, Milford, MA, USA) column were tested. The results indicated that Zorbax ODS C8 column was the most suitable option for the analysis of notoginsenoside Fc and ginsenoside Rc because of good peak resolution and chromatographic separation (Fig. 3). The acetonitrile–water system was the common mobile phase for the separation of ginsenosides. Column temperatures, 25°C and 35°C, were also

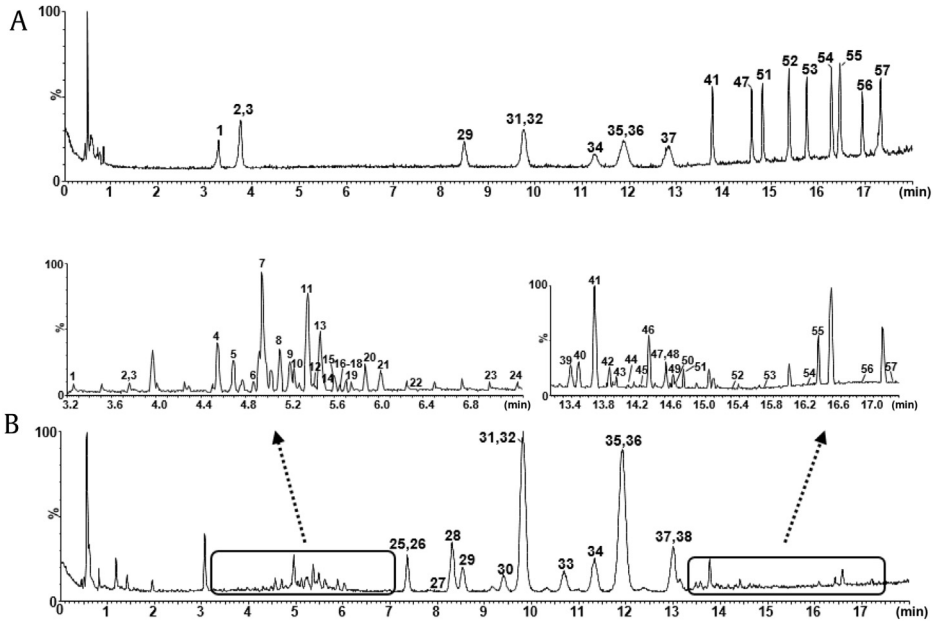

Fig. 4. Total ion chromatograms. (A) Nineteen authentic standards. (B) Methanolic extract of *Panax notoginseng* leaves analyzed by UPLC/Q-TOF MS in negative ion mode. UPLC/Q-TOF MS, ultra-performance liquid chromatography coupled with time-of-flight mass spectrometry.

Table 1
UPLC/Q-TOF MS accurate mass measurements for the constituents in *Panax notoginseng* leaves

No.	Identification	Formula	t_R (min)	Experimental (*m/z*)	Adducts	Error (ppm)	MS^2 fragment ions (*m/z*)
1	Notoginsenoside R_1[1)]	$C_{47}H_{80}O_{18}$	3.30	977.5330	$[M+HCOO]^-$	0.9	931.5229[M-H]$^-$, 799.4821[M-H-Xyl]$^-$, 637.4335[M-H-Xyl-Glc]$^-$, 475.3758[M-H-Xyl-2Glc]$^-$
2	Ginsenoside Re[1)]	$C_{48}H_{82}O_{18}$	3.75	991.5461	$[M+HCOO]^-$	−1.7	945.5411[M-H]$^-$, 799.4902[M-H-Rha]$^-$, 637.4322[M-H-Rha-Glc]$^-$, 475.3774[M-H-Rha-2Glc]$^-$
3	Ginsenoside Rg_1[1)]	$C_{42}H_{72}O_{14}$	3.79	845.4880	$[M+HCOO]^-$	−2.2	799.4853[M-H]$^-$, 637.4276[M-H-Glc]$^-$, 475.3952[M-H-2Glc]$^-$
4	Notoginsenoside R_4	$C_{58}H_{98}O_{27}$	4.56	1,271.6311	$[M+HCOO]^-$	3.1	1,225.6260[M-H]$^-$, 1,093.5817[M-H-Xyl]$^-$, 913.5169[M-H-Xyl-Glc-H_2O]$^-$, 781.4765[M-H-2Xyl-Glc-H_2O]$^-$, 619.4171[M-H-Xyl-2Glc-H_2O-Xyl]$^-$, 475.3736[M-H-2Xyl-2Glc-H_2O -144]$^-$
5	Yesanchinoside H	$C_{53}H_{90}O_{23}$	4.70	1,139.5876	$[M+HCOO]^-$	2.4	1,093.5841[M-H]$^-$, 781.4742[M-H-Xyl-Glc-H_2O]$^-$, 619.4242[M-H-Xyl-2Glc-H_2O]$^-$
6	Floranotoginsenoside A	$C_{53}H_{90}O_{23}$	4.88	1,139.5859	$[M+HCOO]^-$	0.9	1,093.5814[M-H]$^-$, 781.4765[M-H-Xyl-Glc-H_2O]$^-$
7	Floranotoginsenoside D	$C_{53}H_{90}O_{23}$	4.96	1,139.5859	$[M+HCOO]^-$	0.9	1,093.5802[M-H]$^-$, 781.4722[M-H-Xyl-Glc-H_2O]$^-$, 619.4194[M-H-Xyl-2Glc-H_2O]$^-$
8	Floranotoginsenoside B/C/isomer	$C_{53}H_{90}O_{24}$	5.12	1,155.5817	$[M+HCOO]^-$	1.6	1,109.5746[M-H]$^-$;797.4678[M-H-Xyl-Glc-H_2O]$^-$, −635.4171[M-H-Xyl-2Glc-H_2O]$^-$
9	Gypenoside LXIX	$C_{53}H_{90}O_{23}$	5.21	1,139.5861	$[M+HCOO]^-$	1.1	1,093.5800[M-H]$^-$, 961.5390[M-H-Xyl]$^-$, 781.4728[M-H-Xyl-Glc-H_2O]$^-$, 637.4289[M-H-Xyl-2Glc]$^-$, 619.4134[M-H-Xyl-2Glc-H_2O]$^-$, 475.3815[M-H-Xyl-3Glc]$^-$
10	Floranotoginsenoside B/C/isomer	$C_{53}H_{90}O_{24}$	5.25	1,155.5807	$[M+HCOO]^-$	0.7	1,109.5751[M-H]$^-$, 1,091.5656[M-H-H_2O]$^-$, 959.5143[M-H-H_2O-Xyl]$^-$, 797.4699[M-H-H_2O-Xyl-Glc]$^-$, 779.4565[M-H-2H_2O-Xyl-Glc]$^-$, 635.4171[M-H-H_2O-Xyl-2Glc]$^-$, 617.4033[M-H-H_2O-Xyl-2Glc-H_2O]$^-$, 455.3516[M-H-H_2O-Xyl-3Glc-H_2O]$^-$
11	Floranotoginsenoside B/C/isomer	$C_{53}H_{90}O_{24}$	5.37	1,155.5830	$[M+HCOO]^-$	2.7	1,109.5771[M-H]$^-$, 1,091.5608[M-H-H_2O]$^-$, 797.4686[M-H-H_2O-Xyl-Glc]$^-$, 635.4095[M-H-H_2O-Xyl-2Glc]$^-$, 455.3589[M-H-2H_2O-Xyl-3Glc]$^-$
12	Notoginsenoside R_3	$C_{48}H_{82}O_{19}$	5.42	1,007.5419	$[M+HCOO]^-$	−0.8	961.5381[M-H]$^-$, 781.4761[M-H-H_2O-Glc]$^-$, 637.4287[M-H-2Glc]$^-$, 475.3951[M-H-3Glc]$^-$
13	Floranotoginsenoside B/C/isomer	$C_{53}H_{90}O_{24}$	5.48	1,155.5807	$[M+HCOO]^-$	0.7	1,109.5747[M-H]$^-$, 1,091.5662[M-H-H_2O]$^-$, 797.4697[M-H-H_2O-Xyl-Glc]$^-$, 779.4584[M-H-H_2O-Xyl-Glc- H_2O]$^-$, 635.4138[M-H-H_2O-Xyl-2Glc]$^-$, 617.4055[M-H-H_2O-Xyl-2Glc-H_2O]$^-$, 473.3615[M-H-H_2O-Xyl-3Glc]$^-$
14	Gypenoside LXXI	$C_{53}H_{90}O_{23}$	5.52	1,139.5870	$[M+HCOO]^-$	1.8	1,093.5793[M-H]$^-$, 931.4987[M-H-Glc]$^-$, 781.4680[M-H-Glc-Xyl-H_2O]$^-$, 637.4295[M-H-Xyl-2Glc]$^-$, 475.3758[M-H-Xyl-3Glc]$^-$
15	Malonyl-floranotoginsenoside B	$C_{56}H_{92}O_{27}$	5.61	1,195.5770	$[M-H]^-$	1.8	1,109.5726[M-H-Mal]$^-$, 797.4702[M-H-Mal-Glc-Xyl-H_2O]$^-$, 635.4181[M-H-Mal-2Glc-Xyl-H_2O]$^-$, 473.3619[M-H-Mal-3Glc-Xyl-H_2O]$^-$
16	Yesanchinoside G/isomer	$C_{53}H_{88}O_{23}$	5.63	1,137.5709	$[M+HCOO]^-$	1.4	1,091.5642[M-H]$^-$, 929.5220[M-H-Glc]$^-$, 797.4678[M-H-Glc-Xyl]$^-$, 635.4143[M-H-2Glc-Xyl]$^-$, 473.3618[M-H-3Glc-Xyl]$^-$
17	Floranotoginsenoside B/C/isomer	$C_{53}H_{90}O_{24}$	5.66	1,155.5796	$[M+HCOO]^-$	−0.3	1109.5789[M-H]$^-$, 797.4686[M-H-Glc-Xyl-H_2O]$^-$
18	Malonyl-vinaginsenoside R_4	$C_{51}H_{84}O_{22}$	5.68	1,047.5387	$[M-H]^-$	1.1	961.5404[M-H-Mal]$^-$, 799.4850[M-H-Mal-Glc]$^-$, 637.4308[M-H-Mal-2Glc]$^-$,
19	Malonyl-floranotoginsenoside C	$C_{56}H_{92}O_{27}$	5.71	1,195.5774	$[M-H]^-$	2.2	1,109.5741[M-H-Mal]$^-$, 929.5260[M-H-Mal-Glc-H_2O]$^-$, 797.4728[M-H-Mal-Glc-H_2O-Xyl]$^-$, 635.4111[M-H-Mal-2Glc-H_2O-Xyl]$^-$, 455.3495[M-H-Mal-3Glc-2H_2O-Xyl]$^-$
20	Floranotoginsenoside B/C/isomer	$C_{53}H_{90}O_{24}$	5.90	1,155.5850	$[M+HCOO]^-$	−0.6	1,109.5806[M-H]$^-$, 797.4711[M-H-Glc-Xyl-H_2O]$^-$, 635.4175[M-H-2Glc-Xyl-H_2O]$^-$, 473.3650[M-H-3Glc-Xyl-H_2O]$^-$
21	Yesanchinoside G/isomer	$C_{53}H_{88}O_{23}$	6.02	1,137.5756	$[M+HCOO]^-$	0.4	1,091.5684[M-H]$^-$, 959.5264[M-H-Xyl]$^-$, 779.4594[M-H-Xyl-Glc-H_2O]$^-$, 635.4183[M-H-Xyl-2Glc-H_2O]$^-$, 473.3719[M-H-Xyl-3Glc-H_2O]$^-$
22	Malonyl-yesanchinoside G	$C_{56}H_{90}O_{26}$	6.30	1,177.5692	$[M-H]^-$	−0.8	1,091.5658[M-H-Mal]$^-$, 959.5140[M-H-Mal-Xyl]$^-$, 779.4615[M-H-Mal-Xyl-Glc-H_2O]$^-$, 635.4276[M-H-Mal-Xyl-2Glc-H_2O]$^-$, 473.3598[M-H-Mal-Xyl-3Glc-H_2O]$^-$
23	Ginsenoside Ra_0	$C_{60}H_{102}O_{28}$	7.01	1,315.6572	$[M+HCOO]^-$	−1.6	1,269.6510[M-H]$^-$, 1,107.5856[M-H-Glc]$^-$, 945.5368[M-H-2Glc]$^-$, 783.5003[M-H-3Glc]$^-$
24	Notoginsenoside T/D	$C_{64}H_{108}O_{31}$	7.27	1,417.6882	$[M+HCOO]^-$	−2.0	1,371.6833[M-H]$^-$, 1,239.6356[M-H-Xyl]$^-$, 1,107.5991[M-H-2Xyl]$^-$, 945.5431[M-H-2Xyl-Glc]$^-$, 783.4780[M-H-2Xyl-2Glc]$^-$, 621.4407[M-H-2Xyl-3Glc]$^-$, 459.3798[M-H-2Xyl-4Glc]$^-$
25	Notoginsenoside Fa	$C_{59}H_{100}O_{27}$	7.36	1,285.6469	$[M+HCOO]^-$	−1.4	1,239.6388[M-H]$^-$, 1,107.5962[M-H-Xyl]$^-$, 945.5422[M-H-Xyl-Glc]$^-$, 783.4908[M-H-Xyl-2Glc]$^-$, 621.4374[M-H-Xyl-3Glc]$^-$, 459.3841[M-H-Xyl-4Glc]$^-$
26	Notoginsenoside Q	$C_{63}H_{106}O_{30}$	7.41	1,387.6783	$[M+HCOO]^-$	−1.5	1,341.6714[M-H]$^-$, 1,209.6288[M-H-Xyl]$^-$, 1,077.5908[M-H-2Xyl]$^-$, 915.5254[M-H-Xyl-Glc]$^-$, 783.4926[M-H-Xyl-Xyl-Glc-2Xyl]$^-$, 621.4348[M-H-3Xyl-2Glc]$^-$, 459.3862[M-H-3Xyl-3Glc]$^-$
27	Malonyl-notoginsenoside Q	$C_{66}H_{108}O_{33}$	8.01	1,427.6705	$[M-H]^-$	−3.4	1,341.6616[M-H-Mal]$^-$, 1,209.6075[M-H-Mal-Xyl]$^-$, 1,077.5942[M-H-Mal-2Xyl]$^-$, 945.5219[M-H-Mal-3Xyl]$^-$, 783.4813[M-H-Mal-3Xyl-Glc]$^-$
28	Ginsenoside Ra_1	$C_{58}H_{98}O_{26}$	8.32	1,255.6349	$[M+HCOO]^-$	2.1	1,209.6278[M-H]$^-$, 1,077.5867[M-H-Xyl]$^-$, 945.5424[M-H-2Xyl]$^-$, 783.4901[M-H-2Xyl-Glc]$^-$, 621.4360[M-H-2Xyl-2Glc]$^-$, 459.3833[M-H-2Xyl-3Glc]$^-$
29	Ginsenoside Rb_1[1)]	$C_{54}H_{92}O_{23}$	8.54	1,153.6041	$[M+HCOO]^-$	3.0	1,107.5978[M-H]$^-$, 945.5436[M-H-Glc]$^-$, 783.4886[M-H-2Glc]$^-$, 621.4368[M-H-3Glc]$^-$, 459.3857[M-H-4Glc]$^-$
30	Ginsenoside Ra_2	$C_{58}H_{98}O_{26}$	9.41	1,255.6348	$[M+HCOO]^-$	2.0	1,209.6307[M-H]$^-$, 1,077.5867[M-H-Xyl]$^-$, 945.5433[M-H-2Xyl]$^-$, 783.4884[M-H-2Xyl-Glc]$^-$, 621.4340[M-H-2Xyl-2Glc]$^-$, 459.3817[M-H-2Xyl-3Glc]$^-$
31	Notoginsenoside Fc[1)]	$C_{58}H_{98}O_{26}$	9.81	1,255.6362	$[M+HCOO]^-$	3.1	1,209.6302[M-H]$^-$, 1,077.5875[M-H-Xyl]$^-$, 945.5453[M-H-2Xyl]$^-$, 783.4911[M-H-2Xyl-Glc]$^-$, 621.4369[M-H-2Xyl-2Glc]$^-$, 459.3831[M-H-2Xyl-3Glc]$^-$
32	Ginsenoside Rc[1)]	$C_{53}H_{90}O_{22}$	9.82	1,123.5929	$[M+HCOO]^-$	2.6	1,077.5865[M-H]$^-$, 945.5434[M-H-Araf]$^-$, 783.4905[M-H-Araf-Glc]$^-$ 621.4359[M-H-Araf-2Glc]$^-$, 459.3828[M-H-Araf-3Glc]$^-$

(*continued on next page*)

Table 1 (*continued*)

No.	Identification	Formula	t_R (min)	Experimental (*m/z*)	Adducts	Error (ppm)	MS^2 fragment ions (*m/z*)
33	Malonyl-ginsenoside Rc	$C_{56}H_{92}O_{25}$	10.69	1,163.5933	$[M-H]^-$	7.2	1,077.5892[M-H-Mal]$^-$, 945.5471[M-H-Mal-Xyl]$^-$, 783.4926[M-H-Mal-Xyl-Glc]$^-$, 621.4380[M-H-Mal-Xyl-2Glc]$^-$, 459.3847[M-H-Mal-Xyl-3Glc]$^-$
34	Ginsenoside Rb_2[1)]	$C_{53}H_{90}O_{22}$	11.33	1,123.5958	$[M+HCOO]^-$	5.2	1,077.5907[M-H]$^-$, 945.5456[M-H-Arap]$^-$, 783.4923[M-H-Arap-Glc]$^-$, 621.4370[M-H-Arap-2Glc]$^-$, 459.3839[M-H-Arap-3Glc]$^-$
35	Ginsenoside Rb_3[1)]	$C_{53}H_{90}O_{22}$	11.93	1,123.5924	$[M+HCOO]^-$	2.1	1,077.5856[M-H]$^-$, 945.5433[M-H-Xyl]$^-$, 783.4879[M-H-Xyl-Glc]$^-$, 621.4349[M-H-Xyl-2Glc]$^-$, 459.3854[M-H-Xyl-3Glc]$^-$
36	Ginsenoside F_1[1)]	$C_{36}H_{62}O_9$	11.97	683.4384	$[M+HCOO]^-$	2.0	—
37	Vinaginsenoside R_7[1)]	$C_{53}H_{90}O_{22}$	12.84	1,123.5897	$[M+HCOO]^-$	−0.3	1,077.5825[M-H]$^-$, 945.5420[M-H-Xyl]$^-$, 783.4888[M-H-Xyl-Glc]$^-$, 621.4325[M-H-Xyl-2Glc]$^-$, 459.3779[M-H-Xyl-3Glc]$^-$
38	Malonyl-ginsenoside Rb_2	$C_{56}H_{92}O_{25}$	12.99	1,163.5870	$[M-H]^-$	1.8	1,077.5874[M-H-Mal]$^-$, 945.5432[M-H-Mal-Xyl]$^-$, 783.4891[M-H-Mal-Xyl-Glc]$^-$, 621.4367 [M-H-Mal-Xyl-2Glc]$^-$, 459.3838[M-H-Mal-Xyl-3Glc]$^-$
39	Malonyl-ginsenoside Rb_3	$C_{56}H_{92}O_{25}$	13.49	1,163.5883	$[M-H]^-$	2.9	1,077.5868[M-H-Mal]$^-$, 945.5412[M-H-Mal-Xyl]$^-$, 783.4875[M-H-Mal-Xyl-Glc]$^-$, 621.4382[M-H-Mal-Xyl-2Glc]$^-$, 459.3818[M-H-Mal-Xyl-3Glc]$^-$
40	Malonyl-vinaginsenoside R_7	$C_{56}H_{92}O_{25}$	13.58	1,163.5872	$[M-H]^-$	2.0	1,077.5870[M-H-Mal]$^-$, 945.5418[M-H-Mal-Xyl]$^-$, 783.4893[M-H-Mal-Xyl-Glc]$^-$, 621.4379[M-H-Mal-Xyl-2Glc]$^-$, 459.3849[M-H-Mal-Xyl-3Glc]$^-$
41	Ginsenoside Rd[1)]	$C_{48}H_{82}O_{18}$	13.77	991.5475	$[M+HCOO]^-$	−0.3	945.5434[M-H]$^-$, 783.4890[M-H-Glc]$^-$, 621.4362[M-H-2Glc]$^-$, 459.3827[M-H-3Glc]$^-$
42	Malonyl-ginsenoside Rd	$C_{51}H_{84}O_{21}$	13.94	1,031.5414	$[M-H]^-$	−1.3	945.5403[M-H-Mal]$^-$, 783.4910[M-H-Mal-Glc]$^-$, 621.4358[M-H-Mal-2Glc]$^-$, 459.3822[M-H-Mal-3Glc]$^-$
43	Quinquenoside III	$C_{50}H_{84}O_{19}$	14.03	1,033.5587	$[M+HCOO]^-$	0.4	987.5533[M-H]$^-$, 825.5005[M-H-Glc]$^-$, 663.4468[M-H-2Glc]$^-$
44	Ginsenoside Rs_1	$C_{55}H_{92}O_{23}$	14.17	1,165.6024	$[M+HCOO]^-$	1.5	1,119.5950[M-H]$^-$, 987.5580[M-H-Xyl]$^-$, 825.5018[M-H-Xyl-Glc]$^-$, 663.4344[M-H-Xyl-2Glc]$^-$
45	Notoginsenoside P/O	$C_{52}H_{88}O_{21}$	14.31	1,093.5811	$[M+HCOO]^-$	1.5	1,047.5780[M-H]$^-$, 915.5310[M-H-Xyl]$^-$, 783.4874[M-H-2Xyl]$^-$, 621.4375[M-H-2Xyl-Glc]$^-$, 459.3806[M-H-2Xyl-2Glc]$^-$
46	Notoginsenoside R_2	$C_{41}H_{70}O_{13}$	14.42	769.4380	$[M-H]^-$	0.8	637.4329[M-H-Xyl]$^-$, 475.3778[M-H-Xyl-Glc]$^-$
47	Notoginsenoside Fe[1)]	$C_{47}H_{80}O_{17}$	14.62	961.5399	$[M+HCOO]^-$	2.8	915.5349[M-H]$^-$, 783.4913[M-H-Araf]$^-$, 621.4378[M-H-Araf-Glc]$^-$, 459.3846[M-H-Araf-2Glc]$^-$
48	Vinaginsenoside R_{18}	$C_{47}H_{80}O_{17}$	14.66	961.5374	$[M+HCOO]^-$	0.2	915.5333[M-H]$^-$, 783.4916[M-H-Xyl]$^-$, 621.4368[M-H-Xyl-Glc]$^-$, 459.3862[M-H-Xyl-2Glc]$^-$
49	Malonyl-notoginsenoside Fe	$C_{50}H_{82}O_{20}$	14.71	1,001.5342	$[M-H]^-$	2.1	915.5324[M-H-Mal]$^-$, 783.4901[M-H-Mal-Xyl]$^-$, 621.4343[M-H-Mal-Xyl-Glc]$^-$, 459.3830[M-H-Mal-Xyl-2Glc]$^-$
50	Malonyl-vinaginsenoside R_{18}	$C_{50}H_{82}O_{20}$	14.76	1,001.5339	$[M-H]^-$	2.1	915.5336[M-H-Mal]$^-$, 783.5008[M-H-Mal-Xyl]$^-$, 621.4343[M-H-Mal-Xyl-Glc]$^-$, 459.3801[M-H-Mal-Xyl-2Glc]$^-$
51	Notoginsenoside Fd[1)]	$C_{47}H_{80}O_{17}$	14.83	961.5380	$[M+HCOO]^-$	0.8	915.5329 [M-H]$^-$, 783.4910[M-H-Xyl]$^-$, 621.4327[M-H-Xyl-Glc]$^-$, 459.3813[M-H-Xyl-2Glc]$^-$
52	Ginsenoside F_2[1)]	$C_{42}H_{72}O_{13}$	15.39	829.4967	$[M+HCOO]^-$	2.2	783.4921[M-H]$^-$, 621.4332[M-H-Glc]$^-$, 459.3885[M-H-2Glc]$^-$
53	20(*S*)-Ginsenoside Rg_3[1)]	$C_{42}H_{72}O_{13}$	15.76	829.4952	$[M+HCOO]^-$	0.4	783.4907[M-H]$^-$, 621.4308[M-H-Glc]$^-$, 459.3819[M-H-2Glc]$^-$
54	Ginsenoside Mc[1)]	$C_{41}H_{70}O_{12}$	16.29	799.4841	$[M+HCOO]^-$	−0.4	621.4302[M-H-Araf]$^-$, 459.3766[M-H-Araf-Glc]$^-$
55	Gypenoside XIII[1)]	$C_{41}H_{70}O_{12}$	16.47	799.4828	$[M+HCOO]^-$	−2.0	753.4820[M-H]$^-$, 621.4345[M-H-Xyl]$^-$, 459.3931[M-H-Xyl-Glc]$^-$
56	Ginsenoside Rk_1[1)]	$C_{42}H_{70}O_{12}$	16.94	811.4802	$[M+HCOO]^-$	−5.2	—
57	Compound K[1)]	$C_{36}H_{62}O_8$	17.33	667.4420	$[M+HCOO]^-$	−0.1	459.3820[M-H-Glc]$^-$

Ara(p), α-L-arabinose (pyranose); Ara(f), α-L-arabinose (furanose); Glc, β-D-glucose; Mal, malonyl; Rha, α-L-rhamnose; UPLC/Q-TOF MS, ultra-performance liquid chromatography coupled with time-of-flight mass spectrometry; Xyl, β-D-xylose

[1)] Further confirmation by the aid of their authentic standards

compared, and the major peaks in the chromatogram showed the higher resolution and better peak shape at 35°C.

3.3. Optimization of UPLC/Q-TOF MS conditions

To achieve better resolution in short analysis time, two types of chromatographic columns—ACQUITY HSS T3 (100 mm × 2.1 mm i.d., 1.8 μm, Milford, MA, USA) and ACQUITY BEH C18 (100 mm × 2.1 mm i.d., 1.7 μm, Milford, MA, USA)—were used and compared. The results showed that HSS T3 column not only exhibited the better resolution and higher peak capacity, but could also be used to retain and separate the more hydrophilic ginsenosides. Different mobile phases, such as methanol–water and acetonitrile–water systems with diverse modifiers, were compared to obtain the chromatographic separation with the best resolution. The results shown that 0.1% aqueous formic acid–acetonitrile system was the optimum choice. MS data acquired in positive and negative ion modes were compared, and the results suggested that negative ion mode provided the more direct structural information of ginsenosides and higher sensitivities, which was consistent with previous studies [3,14,15]. In order to rapidly acquire the MS/MS fragmentation data for the identification of chemical constituents, the MS^E data acquisition mode was used to simultaneously collect the information from both precursor ion and their related fragment ions in a single run.

3.4. Qualitative analysis of chemical constituents in PNL by UPLC/Q-TOF MS

Nineteen authentic standards and methanolic extract of PNL were determined by UPLC/Q-TOF MS under the optimized conditions, and their typical total ion chromatograms are shown in Fig. 4. A total of 57 saponins were identified in PNL by comparison of retention behavior as well as MS data found in the literature [16–21], and are shown in Table 1. Among them, 19 compounds—ginsenosides Rg1, Rb1, Rb2, Rb3, Rc, Rd, Re, Rg3, F1, F2, Mc, Rk1, and compound K, and notoginsenosides R_1, Fc, Fd, Fe, and gypenoside XIII, vinaginsenoside R7—were also unambiguously characterized by the aid of their authentic standards. In the present study, MS^E acquisition mode, a unique data-independent fragmentation approach, was used to simultaneously collect the information from both precursor ion and their relevant fragment ions. In MS^E mode, MS and MS/MS spectra were acquired by alternating dissociations induced by low-energy and high-energy collision in a single run. This technology provided accurate molecular weight and sufficient MS/MS fragment ions without the need for precursor ion selection. In this study, all compounds, except ginsenosides Rk1 and F1, exhibited abundant MS/MS information using MS^E technology (Table 1), which considerably facilitated the identification of saponins in PNL.

The ionization of ginsenosides offered the intense deprotonated molecular ion $[M\text{-}H]^-$ and solvent adduct ion $[M + HCOO]^-$ in negative ion mode. In fact, the production of adduct ion depends on the modifier added in the mobile phase [22], and 0.1% aqueous formic acid was used as the mobile phase in this study. In MS/MS spectra of the adduct ion, the $[M\text{-}H]^-$ ion was generated from $[M + HCOO]^-$ ion after the loss of one HCOOH unit, which facilitated the confirmation of its deprotonated molecular ion. The common fragmentation behavior of ginsenosides was the simultaneous or successive losses of glycosidic unit until the formation of $[Aglycon\text{-}H]^-$ ions. Based on the structural characteristics of ginsenosides, PTS and PDS generated the aglycon ions at *m/z* 475 and *m/z* 459, respectively. The species and amount of sugar moieties were observed from MS/MS data, in which the mass differences of 146 and 162 suggested the presence of an α-L-rhamnose and β-D-glucose, respectively, and the mass difference of 132 indicated the presence of a pentose [β-D-xylose or α-L-arabinose (pyranose or furanose)]. For example, notoginsenoside Fe (peak 47, Rt = 14.62 min) showed a deprotonated molecular ion $[M\text{-}H]^-$ (*m/z* 915.5349) and an adduct ion $[M\text{-}HCOO]^-$ (*m/z* 961.5399) and with high abundance. The accurate deprotonated molecular weight (*m/z*

Table 2
Calibration curves, linear range, LOD, and LOQ for seven investigated saponins in *Panax notoginseng* leaves

Saponins	RT (min)	Calibration curves[1)]	r^2	Test range (μg/mL)	LOD (μg)	LOQ (μg)
G-Rb_1	22.34	$y = 4 \times 10^6x + 11{,}380$	0.9997	7.50–240	0.213	0.750
NG-Fc	24.47	$y = 3 \times 10^6x + 8{,}780$	0.9998	9.61–307	0.480	0.961
G-Rc	25.63	$y = 3 \times 10^6x + 3{,}157$	0.9999	10.9–350	0.547	0.109
G-Rb_2	29.12	$y = 3 \times 10^6x - 1{,}193$	0.9998	10.8–345	0.920	0.108
G-Rb_3	30.86	$y = 3 \times 10^6x + 20{,}402$	0.9997	37.5–1200	0.938	0.375
NG-Fe	45.87	$y = 4 \times 10^6x + 5{,}018$	0.9996	2.89–92.5	0.723	0.289
NG-Fd	47.23	$y = 4 \times 10^6x + 14{,}293$	0.9990	5.27–168	0.659	0.527

G: ginsenoside; LOD, limit of detection; LOQ, limit of quantification; NG, notoginsenoside; RT, room temperature
[1)] *y*, peak area; *x*, concentration (mg/mL)

Table 3
Precision, reproducibility, and stability data of the established HPLC-UV method

Saponins	Intraday precision (*n* = 6)		Interday precision (*n* = 3)		Reproducibility (*n* = 6)		Stability	
	Mean (μg/mL)	RSD (%)	Mean (μg/mL)	RSD (%)	Mean (μg/mL)	RSD (%)	Mean (μg/mL)	RSD (%)
G-Rb_1	30.22	1.88	30.29	1.61	35.53	2.98	30.39	2.02
NG-Fc	201.74	0.92	200.16	3.05	209.02	2.22	202.37	0.67
G-Rc	282.54	0.95	280.55	2.84	291.10	2.77	283.67	0.91
G-Rb_2	115.85	1.80	114.56	3.54	114.58	1.71	116.34	2.37
G-Rb_3	567.86	0.92	562.04	2.57	588.09	1.97	570.22	0.30
NG-Fe	13.33	2.95	13.21	3.77	13.69	2.77	13.51	3.32
NG-Fd	24.59	3.17	23.59	4.40	24.58	2.49	23.19	4.07

G, ginsenoside; HPLC, high performance liquid chromatography; NG, notoginsenoside; RSD, relative standard deviation

Table 4
Recovery test of the seven saponins in *P. notoginseng* leaves (PNL10 sample)

Saponins	Original (mg)	Spiked (mg)	Found (mg)	Recovery (%)	RSD (%)
G-Rb_1	0.318	0.254	0.573	100.6	0.78
		0.318	0.642	102.1	0.23
		0.381	0.707	102.1	1.86
NG-Fc	0.538	0.430	0.963	98.7	0.92
		0.538	1.093	103.2	1.29
		0.646	1.205	103.3	0.70
G-Rc	0.867	0.694	1.561	100.0	1.01
		0.867	1.763	103.3	1.14
		1.041	1.952	104.3	0.63
G-Rb_2	0.204	0.163	0.367	99.8	0.61
		0.204	0.412	101.9	0.45
		0.245	0.448	99.7	1.74
G-Rb_3	1.635	1.308	2.963	101.5	0.71
		1.635	3.369	106.1	1.24
		1.962	3.668	103.6	1.00
NG-Fe	0.152	0.121	0.276	102.3	1.56
		0.152	0.304	100.3	1.89
		0.182	0.335	100.4	0.83
NG-Fd	0.232	0.186	0.422	101.8	0.87
		0.232	0.462	98.8	2.14
		0.279	0.515	101.1	0.17

G, ginsenoside; NG, notoginsenoside; PNL, *Panax notoginseng* leaves; RSD, relative standard deviation

Table 5
Contents (mg/g) of seven saponins in 10 batches of *Panax notoginseng* leaves ($n = 3$)

Samples	G-Rb_1	NG-Fc	G-Rc	G-Rb_2	G-Rb_3	NG-Fe	NG-Fd
PNL01	1.97 ± 0.11	13.38 ± 0.11	15.30 ± 0.11	5.54 ± 0.05	31.69 ± 0.24	—	—
PNL02	2.45 ± 0.86	8.18 ± 0.24	14.55 ± 0.54	6.65 ± 0.25	30.82 ± 1.13	—	—
PNL03	2.12 ± 0.08	11.36 ± 0.15	14.39 ± 0.34	6.44 ± 0.10	29.02 ± 0.47	0.25 ± 0.01	0.50 ± 0.04
PNL04	1.66 ± 0.17	12.51 ± 0.05	12.26 ± 0.25	4.99 ± 0.01	23.88 ± 0.29	—	—
PNL05	2.34 ± 0.20	11.94 ± 0.04	16.30 ± 0.22	6.71 ± 0.07	32.60 ± 0.47	0.20 ± 0.01	0.35 ± 0.03
PNL06	2.48 ± 0.01	11.73 ± 0.12	14.90 ± 0.16	5.98 ± 0.06	28.58 ± 0.50	—	—
PNL07	2.30 ± 0.02	11.90 ± 0.07	13.97 ± 0.08	5.25 ± 0.07	25.44 ± 0.16	0.27 ± 0.01	0.42 ± 0.01
PNL08	2.31 ± 0.02	12.14 ± 0.10	14.25 ± 0.07	5.97 ± 0.22	29.86 ± 0.37	—	—
PNL09	3.08 ± 0.07	11.90 ± 0.08	14.85 ± 0.15	5.81 ± 0.07	27.51 ± 0.31	—	—
PNL10	1.44 ± 0.02	9.47 ± 0.08	14.08 ± 0.11	4.81 ± 0.10	28.23 ± 0.04	0.58 ± 0.01	0.98 ± 0.01

—, under limit of quantification; G, ginsenoside; NG, notoginsenoside; PNL, *Panax notoginseng* leaves

915.5349) means that its empirical molecular formula was $C_{47}H_{80}O_{17}$. In the MS/MS of the adduct ion at *m*/*z* 961.5399 (Fig. S2), the $[M-H]^-$ ion, along with three major fragment ions at *m*/*z* 783.4913, 621.4378, and 459.3846, were observed. The mass differences between the parent ion and product ions at *m*/*z* 783.4913 and *m*/*z* 621.4378 were 132 and 162, respectively, attributed to the loss of one pentose unit and successive loss of one glucose unit. The fragment ion at *m*/*z* 459.3846 indicated PDS aglycon moiety losing all linked glycosidic units. By the aid of authentic standard, the chemical structure of Peak 47 was unambiguously identified as notoginsenoside Fe.

3.5. Validation of HPLC-UV method

As shown in Table 2, the correlation coefficient ($r^2 > 0.999$) indicated good correlations between their peak areas and the concentrations of saponins concerned. LOD and LOQ for all investigated saponins were less than 98 ng and 375 ng on column, respectively. Overall inter- and intraday precisions were less than 4.40% and 3.17%, respectively, for all seven saponins (Table 3). The tested saponins were relatively stable with an overall variation of 0.30–4.07% at least 6 h at room temperature. The repeatability of the analytes concerned in the test sample (PNL10) was acceptable with a relative standard deviation of <2.98%. In addition, the developed method had a good accuracy with an overall recovery of 98.7–106.1% (Table 4).

3.6. Quantitation of seven major saponins in PNL by HPLC-UV

The developed HPLC-UV method was applied to quantitative determination of the seven saponins in 10 batches of PNL samples. As shown in Table 5. The results showed that the contents of ginsenosides Rb1, Rc, Rb2, and Rb3, and notoginsenoside Fc, had no significant difference among the 10 samples. Interestingly, a small number of notoginsenosides Fe and Fd were detected in four batches of PNL, and their contents were below the LOQ in six samples. A most plausible explanation is that these samples, containing notoginsenosides Fe and Fd, might be exposed to moisture during storage or transportation, considering that ginsenosides Rc and Rb_3 might be transformed into notoginsenosides Fe and Fd, respectively, by enzymatic hydrolysis in the present of water.

In conclusion, UPLC/Q-TOF MS and HPLC-UV methods were developed for the qualitative and quantitative analysis of the saponins in PNL, respectively. For the qualitative analysis, a total of 57 saponins were identified in PNL; among them, 19 components were unambiguously characterized by comparing with their authentic standards. For the quantitative analysis, seven saponins were simultaneously determined in 10 batches of PNL using HPLC-UV, which has good precision, accuracy, and sensitivity. Additionally, our results also demonstrated that ginsenosides Rc and Rb3, two major saponins of PNL, can easily be transformed to notoginsenosides Fe and Fd, respectively, by removing a glucose residue from position C-3 via enzymatic hydrolysis when water was used as the extraction solvent, suggesting that efforts should be made to ensure that *P. notoginseng* leaves are not exposed to moisture during storage or transportation. These results are beneficial to the quality control of PNL and its relevant products.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jgr.2017.01.007.

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